**Is electrolyte transfer across the urothelium important?**

Karen D McCloskey*[1], Bahareh Vahabi[2] and Christopher H Fry[2],

[1.]Centre for Cancer Research and Cell Biology, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, BT9 7AE

[2.] School of Physiology, Pharmacology and Neuroscience, University of Bristol, BS8 1TD.

Author for correspondence*
Karen D McCloskey PhD
[.]Centre for Cancer Research and Cell Biology,
School of Medicine, Dentistry and Biomedical Sciences,
Queen's University Belfast,
Belfast, BT9 7AE
k.mccloskey@qub.ac.uk

Abstract
AIMS

This article summarizes discussion at the International Consultation on Incontinence Research Society (ICI-RS) 2015 meeting of urine modification in the urinary tract by the urothelium. It considers the literature and proposes pertinent questions that need to be addressed to understand this phenomenon within a physiological context.

METHODS

Following the ICI-RS meeting, publications in PubMed relating to urine modification in the renal pelvis, ureter, and bladder were reviewed.

RESULTS

Historically, the urothelium has been simply considered as a passive, impermeable barrier, preventing contact between urine and the underlying cells. In addition to the ability of the umbrella cells to modify the surface area of the urothelium during bladder filling, the urothelium may also be involved in modifying urine composition. Several lines of evidence support the hypothesis that electrolytes and water can be reabsorbed by the urothelium and that this may have physiological relevance. Firstly, urothelial cells express several types of aquaporins and ion channels; the membrane expression of which is modulated by the extracellular concentration of ions including Na+. Secondly, studies of urine composition in the renal pelvis and bladder demonstrate urine modification, indicating that water and/or electrolyte transport has occurred. Thirdly, hibernating mammals, with urothelial and bladder wall histology similar to non-hibernating mammals are known to produce and reabsorb urine daily, during long periods of hibernation.

CONCLUSIONS

The phenomenon of urine modification by the urothelium may be physiologically important during normal bladder filling. Research should be focused on investigating how this may change in conditions of urinary dysfunction.

## INTRODUCTION

The urinary tract comprising renal pelvis, ureter, bladder, and urethra has traditionally been regarded as a conduit and storage system for urine before it is expelled from the body during voiding. The epithelial lining of the bladder, known as the urothelium, provides a high-resistance barrier between the urine and the cells/interstitium of underlying tissues, yet, there is substantial evidence that the urothelium represents a dynamic tissue with additional physiological functions.[1, 2] The urothelium is now known to be important in the transduction of information on, for example, bladder fullness to the underlying lamina propria, mucosal neuronal plexus, and the underlying detrusor smooth muscle.[3] The urothelium typically comprises large umbrella (surface) cells, up to 250 μm diameter, several layers of intermediate cells, and a layer of basal cells on a basement membrane. Much is known of the ultrastructural properties of the urothelium including the molecular identity and the physiological roles of structural and intercellular proteins, reviewed extensively elsewhere.[1, 2, 4] The luminal umbrella cells face a unique challenge with their basolateral surfaces exposed to plasma which has a different composition to the urine which bathes the apical luminal surface. These cells have the crucial role of increasing the urothelial surface area during bladder filling through insertion of membrane from apical membrane endosomes followed by endocytosis after voiding. In addition, it is known that uroplakin proteins (UP) on umbrella cells are involved in the pathogenesis of urinary infections, for example, UP1a which is co-opted by uropathogenic bacteria as a receptor enabling cellular adhesion and subsequent colonization.[2]

The urothelium is increasingly recognized as having the potential to modify the composition of stored urine.[5, 6] This is counter-intuitive to its role as a passive barrier where it is assumed to prevent contact between the urine and urinary tract cells, thereby preserving urine composition. This is the essential basis of clinical urinalysis where urine expelled from the bladder is considered as being representative of urine excreted by the kidney. Yet, it has been known for several decades that the bladder, renal pelvis,[7] and ureters[8] have the potential to modify urine via transport of $Na^+$, $K^+$, urea, water, and creatinine across the urothelium.[9] Furthermore, the $Na^+$-conserving hormone, aldosterone, increases Na+ transport across the bladder wall[10] and anti-diuretic hormone (ADH) induces net movement of water across the frog bladder wall.[11]

The aim of the present article is to review what is known of urine modification in the urinary tract and to highlight areas of research needed to advance our understanding of this area within a clinically relevant context.

## 1 FUNCTIONAL EXPRESSION OF AQUAPORINS IN UROTHELIUM

Aquaporins (AQP) are integral membrane proteins forming pores, which transport water, glycerol, and some solutes.[12] AQPs are known to be involved in fluid homeostasis, water transport, glycerol, and fat transport in kidney epithelial cells, vascular endothelial cells, adipocytes, and epidermal cells. At least seven AQPs, AQP-1, 2, 3, 4, 6, 7, 8, are functionally expressed in the kidney for water transport and urine concentration.[13] Their somewhat surprising expression in urothelial cells suggests that these cells may also be capable of water transport in the urinary tract, particularly the bladder. Gene expression of AQP-3, 4, 7, 9, and 11 has been demonstrated in fresh human urothelium, moreover, protein expression of AQP-3, 4, 7, and 9 was confirmed with immunofluorescence.[14] AQP-3 protein

has also been reported in human bladder from tissue microarray immunohistochemistry studies.[15] Recently, AQP-1, 3, 9, and 11 have been identified in another mammalian model, pig bladder (B. Vahabi, M. Manso, M. Drake, C.H. Fry, unpublished data). Interestingly, the urothelium of the American black bear, a mammal that typically hibernates for 4–5 months and does not eat, drink, urinate, or defecate during that time but is known to produce and reabsorb urine in equal amounts daily,[16] also expresses AQP-1 and AQP-3.[17]

The cellular localization of AQPs in urothelial cells is an interesting indicator of functionality. AQP-2 and AQP-3 are located on the basolateral (not apical) membrane of umbrella cells and the plasma membranes of neighboring intermediate and basal cells in rat bladder.[18] A similar distribution of AQP-3 has been reported for dog bladder urothelium.[19] It has been proposed that specific AQPs are expressed on the apical membrane of umbrella cells, for initial water influx and subsequent egression through basolateral AQPs, for example, AQP-2 or AQP-3 to the intermediate and basal cells.[2] This would appear to be an elegant mechanism for water transport from the bladder lumen, through the urothelial cell layers toward the interstitial space and capillaries in the lamina propria.

Functional experiments using a model of in vitro differentiated normal human urothelial cell cultures showed that expression of AQP-3 and AQP-9 was upregulated by increased osmolality through changes in NaCl concentration. Interestingly, blockade of AQPs with mercuric chloride reduced water and urea flux showing AQP functionality under these conditions,[20] consistent with urothelium sensing and responding to hyperosmotic conditions to maintain water and salt homeostasis. In vivo experiments on rats in conditions of water deprivation or water loading showed that expression of AQP-2 and AQP-3 was significantly increased in the water deprivation conditions. The above studies are consistent with the hypothesis that AQPs impact urine osmolality through water transport under conditions of water deprivation/dehydration.

It is interesting to note that roles of AQPs other than water transport may also occur in the urothelium. The ability of AQPs to transport cations may also contribute to urine modification, moreover, their regulation of the expression of other proteins, involvement in cell adhesion, and control of cell volume[21] suggest extensive involvement in urothelial physiology.

## 3 ION CHANNELS ON UROTHELIAL CELLS FOR ELECTROLYTE TRANSPORT

Urothelial cells express a large number of ion channels and transport proteins, in keeping with the ability to modify urine ionic composition and osmolality by absorption or secretion. These have been reviewed by others[1, 2, 4, 22-24] and will be briefly summarized here.

### 3.1 ENaC and $Na^+/K^+$-ATPase

The epithelial sodium channel, ENaC, also known as the amiloride-sensitive sodium channel is expressed on the apical membrane of umbrella cells, facilitating transurothelial $Na^+$ flux along with $Na^+/K^+$-ATPase on the basolateral membrane, which provides the driving force for $Na^+$ absorption. In vitro preparations of urothelium in Ussing chambers in experimental conditions to mimic bladder filling exhibit significant increased ENaC activity, potentially from apical membrane incorporation of ENaCs via vesical fusion.[25, 26] Such findings

demonstrate the mechanisms available for $Na^+$-transport from the urine, across the urothelial layer, eventually to lamina propria capillaries. Determining whether this occurs in vivo in healthy bladders during filling represents a challenge. The use of transgenic models, for example, with conditional urothelial ENaC knockdown, would be an interesting approach enabling dissection of the involvement of ENaC-mediated Na+-absorption and its impact on urine composition.

### 3.2 $K^+$ channels

Several mechanisms for $K^+$ transport are reportedly present in urothelial cells including non-selective cation channels (NSCC) expressed on the apical membrane. NSCC channels are mechanosensitive and also transport $Ca^{2+}$ across the apical surface of umbrella cells. Their molecular identity is not yet certain. Other $K^+$ channels in the urothelium include Kir1.1 (inwardly rectifying $K^+$ channel), which is reportedly expressed on umbrella cells' apical membrane[27] and the Kir2.1 channel, also known as the heparin-binding EGF channel. While the function(s) of Kir1/Kir2.1 channels in umbrella cells are not fully known, they may be involved in modification of urinary [$K^+$]. Members of the $Ca^{2+}$-activated potassium channel family are also expressed in urothelium; gene expression of the large-conductance $Ca^{2+}$-activated $K^+$ channel (KCNMA1, BK channel) and the small/intermediate-conductance $Ca^{2+}$-activated $K^+$ channel (KCNN1-4, SK/IK) have been reported.28 In an earlier study, Wang et al.[26] reported that the SK channel blocker apamin, and the BK/IK blocker, charybdotoxin reduced $K^+$ transport, whereas the selective BK blocker, iberiotoxin had no effect; overall indicating that SK and IK but not BK channels functionally transport $K^+$ in urothelial cells. In the same study, contribution of ATP-sensitive $K^+$-channels (KATP) to $K^+$ secretion from urothelial cells was shown by reduction of $K^+$-transport with the KATP blocker, glibenclamide. Consistent with this, gene expression of the KATP channels, Kir6.1 (KCNJ8) and Kir6.2 (KCNJ11) and protein expression of Kir6.1 at the basolateral membrane of umbrella cells and on other urothelial cells has been reported.[28]

### 3.3 TRP channels

TRP channels are non-selective cation channels permeable to calcium, magnesium and sodium. Several of these are known to be expressed by urothelial cells (reviewed in ref.23) and information on their physiological roles is emerging. Expression of TRPV1 on urothelial cells is still considered to be putative; similarly, the cold-sensing channel TRPM8 is not widely accepted as being expressed in the urothelium. Everaerts et al.[29] characterized TRPV2, TRPV4, and TRPM7 in mouse urothelium. TRPV2 and TRPV4 are known to be expressed on basal urothelial cells; the latter is located close to adherens junctions and is involved in mechanosensation. Urothelium sensing of bladder fullness or extension is also achieved by novel mechanosensitive Piezo1 channels which transduce increase of cytosolic $Ca^{2+}$-concentrations to ATP release.[30] We do not currently know under which conditions TRP channels are involved in the transurothelial transport of cations; however, TRPV5 and TRPV6 are important mechanisms of $Ca^{2+}$-transport in the epithelial cells of the renal distal convoluted tubule.[31, 32]

### 3.4 Purinergic receptors

A further family of non-selective cation channels is represented by the purinergic, P2X receptors, P2X1-P2X7, all of which have been identified in the urothelium of several species. P2X5, P2X6, and P2X7 are reportedly to be expressed in rat bladder urothelial cells.[33] Vial & Evans[34] demonstrated P2X2, P2X4, and P2X7 on mouse bladder urothelium, moreover all P2Xs were found to be expressed in feline bladder urothelium.[35] P2X1, P2X2, P2X3, P2X6, and P2X7 are expressed on human urothelial cells.[36-39] The potential involvement of P2X receptor/channels in ion transport in the context of urine modification is unknown; however, it is known that P2X receptors on umbrella cells may contribute to apical membrane insertion triggered by $Ca^{2+}$-influx.[40] Given that delivery of ENaCs to the apical membrane occurs via a similar mechanism, P2X-mediated $Ca^{2+}$-influx may indirectly enable $Na^{+}$-influx under conditions where ATP is released from urothelial cells, for example, during filling.

### 3.5 $Cl^{-}$ channels

Secretion of $Cl^{-}$ from urothelial cells has been shown in experimental conditions, yet the molecular identity of the ion channel(s) remains unknown. Wang et al.[26] subsequently discussed pathways for $Cl^{-}$ entry across the basolateral membrane of umbrella cells including $Cl^{-}$ channels and $Cl^{-}$/bicarbonate exchangers but noted that an apical pathway of $Cl^{-}$secretion had not been described in the urothelium.

### 3.6 Solute transporters

Briefly, transporters are present in urothelial cells for urea and creatinine,[2] enabling reabsorption or secretion depending on the local environment. The urea transporter A, UT-A, is located apically, whereas UT-B is primarily located on the basolateral surface of umbrella cells and the membrane of intermediate and basal cells enabling a system of urea absorption apically by UT-A, transport through the cell and exit on the basolateral side via UT-B.

## 4 MEASUREMENT OF URINE ELECTROLYTE CONCENTRATIONS IN ANIMALS AND HUMANS

The concept of ion and water transport across urothelial tissue has been demonstrated in both animal and human models. $Na^{+}$ transport across ex vivo rabbit urothelial tissue[41] and water permeability of human bladder[42] were demonstrated several decades ago. Investigation of the permeability of intact rabbit bladder urothelium by Negrete et al.[43] showed that this was low under normal conditions, consistent with the urothelium acting as an effective barrier. In vivo studies of animals under conditions of water deprivation or water loading have also been employed to investigate the permeability of the bladder to water and several ions. Furthermore, studies of urine composition in patients undergoing renal procedures have provided physiological information on modification of urine as it travels from the kidney to the bladder.

An interesting study of rats during water deprivation, water loading, or ad libitum H2O involved urine collection and its subsequent instillation into isolated in situ bladders from animals in the corresponding condition. Measurements of Na+, K+, and Cl− indicated that (1) the instilled urinary ion concentrations and (2) animal hydration status influenced the

degree of water reabsorption across the urothelium leading the authors to conclude that urine composition was modified in the lower urinary tract.43

Studies of the American black bear, a mammal that typically hibernates for 4–5 months and does not eat, drink, urinate, or defecate during that time,[16] may provide a helpful perspective. The fact that the bear produces urine daily without urination indicates that urine is produced and reabsorbed in similar quantities. The composition of the bladder wall of the black bear was reportedly remarkably similar in histology and ultrastructure to that of non-hibernating mammals including humans. Interestingly, expression of urothelial proteins including uroplakins, occludin, and claudin was similar to other mammals, moreover, expression and localization of AQP-1, AQP-3, and $Na^+/K^+$-ATPase were not remarkably different.[17] These observations coupled with the fact that the bear bladder also operates a filling/emptying cycle in non-hibernating conditions raises the possibility that under certain (patho) physiological conditions, the human bladder might also modify urine composition. Spector et al.[17] noted that it is challenging to obtain bladder tissue from bears during the hibernation period, therefore limiting our ability to determine the functional expression of urothelial proteins and transport mechanisms during this time. They speculated that dietary changes (known to change expression of uroplakins, tight junctions, and ion channels) prior to hibernation, significantly increased consumption of food and subsequent storage of fat may cause upregulation of AQPs and ion channels/transporters and downregulation of the membrane barrier proteins, which together would facilitate urine reabsorption during hibernation.

Evidence for post-kidney urine modification in humans was reported by Cahill et al.[44] who compared urine composition from the renal pelvis and bladder in patients undergoing renal procedures. Measurements of urine pH, osmolality, $Na^+$, and $K^+$ revealed that these differed between bladder and renal pelvis, supporting the hypothesis that urine was modified in the human lower urinary tract in vivo. Recently, an unpublished study of paired urine samples from renal pelvis and bladder taken from 21 well-hydrated patients undergoing percutaneous nephrolithotomy or ureteric stent insertion prior to lithotripsy corroborates the findings of Cahill et al.[44] Samples were analyzed for [$Na^+$] and [$K^+$] with flame photometry; osmolality with an automatic micro-osmometer; and pH with a combination glass electrode. Urine osmolality was increased by a median of 1.6-fold in bladder compared to renal pelvis samples. $Na^+$ and $K^+$ were similarly increased in bladder samples with 1.4-fold and 1.9-fold increases, respectively. The initial, renal pelvis [Na]:[K] was about 4.0, indicating that approximately twice the quantity of Na+ is absorbed compared to $K^+$. Bladder urine pH was around 0.35 pH units less acidic compared to that from renal pelvis; however, the alkaline shift was greater as renal pelvis' acidity increased, and at approximately pH 7.0, pH was unchanged in the two compartments. This suggests a net acid transporter across the urothelium under most conditions. The increase of [$Na^+$] and [$K^+$] in bladder compared to renal pelvis urine suggests significant movement of free water compared to electrolytes across the urinary tract urothelium. Although urine is stored in the bladder for long periods of time, the large surface-to-volume ratio of the ureteric lumen may enable it to have a significant role in regulating water transport. The particular routes for water movement require evaluation; paracellular or transcellular; as do the pathways that regulate such a flux. However, net $Na^+$ and $K^+$ transport is also likely as bladder urine [$K^+$] increases more than [$Na^+$], suggesting preferential electrolyte transport routes.

## 5 PHYSIOLOGICAL AND CLINICAL RELEVANCE

Physiologically, the movement of both water and electrolytes across the urinary tract urothelium indicates a supplementary route to conserve these important moieties and also allows bladder filling not to become excessive with long micturition intervals. It also advises caution in that measurement of bladder urine electrolyte concentrations is not a pure measure of renal function, but also reflects (upper and lower) urinary tract activity. A better understanding of the mechanisms involved in mediating water and solute transport across urinary tract urothelium should aid development of novel therapeutic interventions for treatment of debilitating conditions such as nocturia and overactive bladder syndrome (OAB). Urine concentration and volume is known to affect urge sensation of bladder fullness in patients with lower urinary tract disorders and together may initiate sensory information at level of the urothelium.[44]

## 6 FUTURE RESEARCH PRIORITIES

Several pertinent questions need to be addressed to enable us to understand the clinical relevance of urine modification by the urinary tract at the level of the urothelium and the conditions under which this occurs.

Does urine modification occur physiologically at baseline levels, representing normal homeostasis of water and particular ions?

In mild dehydration, for example, during exercise or fasting prior to surgery, is there upregulation of urothelial permeability to water and ions?

Are compensatory mechanisms for “baseline” modification evoked after urothelium damage, for example, from bacterial infection, spinal cord injury, radiation therapy?

Is urine modification downregulated or augmented in lower urinary tract disease? If so, is it a cause or consequence of lower urinary tract symptoms?

Can urine modification mechanisms be harnessed to mitigate bladder sensation?

Does salt and water movement occur primarily across the bladder wall or also across the wall of the ureters?

Clearly, the development of appropriate experimental platforms including both *ex vivo* cellular systems and *in vivo* models is necessary to advance this area. A major challenge will lie with investigation of physiological baseline urinary modification and elucidating the conditions which change this with pathophysiological effects in patients. In animal models, advanced ion-selective imaging techniques and implantation of micro-scale ion-sensing electrodes at the level of the renal pelvis and bladder seems to be feasible in the short-term. Such an approach in animal models of lower urinary tract disease would be a powerful means of understanding the extent to which urine modification contributes to lower urinary tract symptoms.

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